JC05 Rec'd PCT/PTO 1 8 MAY 2001

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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 118.13-US-WO

U S APPLICATION NO (If known, see 37 CFR 1.5): 09/856114

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/KR99/00699	19 November 1999 (19.11.99)	19 November 1998 (19.11.98)

TITLE OF INVENTION: HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF HBV AND PREPARATION METHOD THEREOF

APPLICANT(S) FOR DO/EO/US: Hyo Jeong Hong, Chun Jeih Ryu, and Hyangsuk Hur

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [X] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information:
    (a) Computer Readable and Paper Form of Sequence Listing

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/856114	PCT/KR99/00699	118.13-US-WO

21. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
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Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	15 - 20 =		x **$18.00**	$
Independent claims	6 - 3 =	3	x **$80.00**	$ 240.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$
TOTAL OF ABOVE CALCULATIONS =				$ 1240.00
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$
SUBTOTAL =				$ 1240.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 1240.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 40.00
TOTAL FEES ENCLOSED =				$ 1280.00
			Amount to be refunded:	$
			charged:	$

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0494 . A duplicate copy of this sheet is enclosed.

d. ☒ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Gates & Cooper LLP
6701 Center Drive West, Suite 1050
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SIGNATURE

Karen S. Canady
NAME

39,927
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Hyo Jeong Hong et al.	Examiner:	To be assigned
Serial No.:	To be assigned	Group Art Unit:	To be assigned
Filed:	To be assigned	Docket:	118.13-US-WO
Title:	HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF HBV AND PREPARATION METHOD THEREOF		

CERTIFICATE OF MAILING UNDER 37 CFR 1.10
'Express Mail' mailing label number: EL816010064US
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I hereby certify that this paper or fee is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

By: Darlene Ross
Name: Darlene Ross

PRELIMINARY AMENDMENT

BOX PCT
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to a first Office Action, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please amend the specification as follows:

At page 1, after the Title, please insert the following paragraph:

--This application claims the benefit of Korean Patent Application No. 1998-49663, filed November 19, 1998.---

IN THE CLAIMS

Please cancel claims 1-15 without prejudice, and add new claims 16-30 as follows:

16. (NEW) A humanized antibody specific for HBV surface antigen pre-S1, containing a humanized heavy chain variable region which comprises an amino acid sequence described by SEQ ID NO: 20.

17. (NEW) A humanized antibody specific for HBV surface antigen pre-S1, containing a humanized heavy chain variable region which comprises an amino acid sequence described by SEQ ID NO: 21.

18. (NEW) The humanized antibody of claim 17, wherein the amino acid sequence of the humanized heavy chain variable region is modified from SEQ ID NO: 21 by at least one amino acid substitution selected from the group comprising:

$Lys^{12} \rightarrow Val^{12}$, $Thr^{28} \rightarrow Ala^{28}$, $Thr^{30} \rightarrow Ser^{30}$,

$Met^{48} \rightarrow Ile^{48}$, $Arg^{67} \rightarrow Lys^{67}$, $Val^{68} \rightarrow Ala^{68}$,

$Met^{70} \rightarrow Leu^{70}$, $Val^{79} \rightarrow Ala^{79}$, and $Tyr^{95} \rightarrow Phe^{95}$.

19. (NEW) A humanized antibody specific for HBV surface antigen pre-S1, containing a humanized light chain variable region which comprises an amino acid sequence described by SEQ ID NO: 23.

20. (NEW) A gene encoding a humanized heavy chain which comprises a humanized heavy chain variable region having an amino acid sequence selected from the group consisting of: the amino acid sequence described by SEQ ID NO: 20; the amino acid sequence described by SEQ ID NO: 21; and the amino acid sequence of SEQ ID NO: 21 which is modified by at least one amino acid substitution

selected from the group comprising: $Lys^{12} \rightarrow Val^{12}$, $Thr^{28} \rightarrow Ala^{28}$, $Thr^{30} \rightarrow Ser^{30}$, $Met^{48} \rightarrow Ile^{48}$, $Arg^{67} \rightarrow Lys^{67}$, $Val^{68} \rightarrow Ala^{68}$, $Met^{70} \rightarrow Leu^{70}$, $Val^{79} \rightarrow Ala^{79}$, and $Tyr^{95} \rightarrow Phe^{95}$.

21. (NEW) The gene of claim 20, wherein the humanized heavy chain variable region comprises an amino acid sequence described by SEQ ID NO: 20.

22. (NEW) The gene of claim 20, wherein the humanized heavy chain variable region comprises an amino acid sequence described by SEQ ID NO: 21.

23. (NEW) A gene encoding a humanized light chain which contains a humanized light chain variable region comprising an amino acid sequence described by SEQ ID NO: 23.

24. (NEW) An expression vector containing the gene of claim 20.

25. (NEW) The expression vector of claim 24, which is designated pCMV-HKR127HC (Accession Number: KCTC 0531BP).

26. (NEW) The expression vector of claim 24, which is designated pCMV-HKR127(III)HC (Accession Number: KCTC 0691BP).

27. (NEW) An expression vector containing the gene of claim 23.

28. (NEW) The expression vector of claim 27, which is designated pKC-dhfr-HKR127 (Accession Number: KCTC 0529BP).

29. (NEW) A pharmaceutical composition containing a humanized antibody specific for HBV surface antigen pre-S1, wherein the humanized antibody comprises:

(a) a humanized heavy chain variable region which comprises an amino acid sequence described by SEQ ID NO: 20,

(b) a humanized heavy chain variable region which comprises an amino acid sequence described by SEQ ID NO: 21;

(c) a humanized heavy chain variable region which comprises an amino acid sequence of SEQ ID NO: 21 which is modified by at least one amino acid substitution selected from the group comprising: $Lys^{12} \rightarrow Val^{12}$, $Thr^{28} \rightarrow Ala^{28}$, $Thr^{30} \rightarrow Ser^{30}$, $Met^{48} \rightarrow Ile^{48}$, $Arg^{67} \rightarrow Lys^{67}$, $Val^{68} \rightarrow Ala^{68}$, $Met^{70} \rightarrow Leu^{70}$, $Val^{79} \rightarrow Ala^{79}$, and $Tyr^{95} \rightarrow Phe^{95}$, or

(d) a humanized light chain variable region comprising an amino acid sequence described by SEQ ID NO: 23

30. (NEW) A method of protecting against HBV infection or treating chronic hepatitis B, comprising administering the pharmaceutical composition of claim 29.

REMARKS

Prior to a first Office Action in this application, Applicants request that original claims 1-15 be canceled without prejudice, and that new claims 16-30 be added. These new claims do not involve

any new matter or objectionable changes. When the Examiner takes this application up for action, it is requested that the foregoing be taken into account.

It is submitted that this application is now in good order for allowance and such allowance is respectfully solicited. Should the Examiner believe minor matters still remain that can be resolved in a telephone interview, the Examiner is urged to call Applicants' undersigned attorney.

Respectfully submitted,

Hyo Jeong Hong et al.

By their attorneys,

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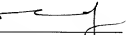
Date: May 18, 2001

By: 
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KSC/dr
G&C 118.13-US-WO

HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF HBV AND PREPARATION METHOD THEREOF

FIELD OF THE INVENTION

The present invention relates to humanized antibodies specific for HBV surface antigen pre-S1.

Particularly, this invention relates to humanized antibodies specific for HBV surface antigen pre-S1, the antibody comprising humanized heavy and light chain; to genes encoding the humanized heavy or light chain; to expression vectors containing said genes and *E. coli* transformants containing said expression vector; and to pharmaceutical composition comprising said humanized antibody, which may be administered in order to prevent HBV infection or to treat chronic hepatitis B.

BACKGROUND

HBV (Hepatitis B Virus) is responsible for chronic or acute human hepatitis that may get worse to liver cirrhosis or cancer. It is estimated that about three hundred million people are suffering from hepatitis in the world (Tiollais and Buendia, *Sci. Am.* 264:48, 1991).

There are three kinds of HBV surface proteins containing different sets of surface antigens. Particularly, these surface antigen proteins includes the Major Protein containing S antigen, the Middle

Protein containing S and pre-S2 antigens, and the Large Protein containing S, pre-S2 and pre-S1 antigens (Neurath and Kent, *Adv. Virus Res.*, 34:65-142, 1988). All the surface antigen proteins can induce antibodies that neutralize HBV, and especially, antibodies against HBV pre-S antigen are associated with the elimination of the virus and the recovery from HBV infection, overcoming non-responsiveness to the S antigen (Iwarson et al., *J. Med. Virol.*, 16:89-96, 1985; Itoh *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:9174-9178, 1986; Budkowska *et al.*, *J. Med. Virol.*, 20:111-125, 1986; Milich *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:8168-8172, 1985; Milich *et al.*, *J. Immunol.*, 137:315-322, 1986).

Unlike pre-S2 or S antigen, pre-S1 antigen is exclusively present in infectious virus particles (Heerman *et al.*, *J. Virol.*, 52:396-402, 1984) and involved in the infection into human liver cells. Thus, it has been reported that monoclonal antibody specific for pre-S1 antigen may efficiently neutralize HBV (Neurath *et al.*, *Cell*, 46:429, 1986; Pontisso *et al.*, *Virology*, 173:533, 1989; Neurath *et al.*, *Vaccine*, 7:234, 1989), and the monoclonal antibody is considered to be useful in the prevention of HBV infection and the treatment of chronic hepatitis B.

So far hepatitis B immunoglobulin has been employed as a preventive for HBV infection, which may

protect, for example, a newborn baby from a HBV-positive mother, medical personnel exposed to HBV, and liver transplant patient with chronic HBV-related liver disease (Beasley *et al., Lancet*, 2:1099, 1983; Todo *et al., Hepatology*, 13:619, 1991). However, hepatitis B immunoglobulin has some shortcomings such as its limited availability, low specific activity and its possible contamination with infectious agents. Furthermore, it is another disadvantage of hepatitis B immunoglobulin that blood plasma should be continuously supplied.

As an alternative for the hepatitis B immunoglobulin, mouse monoclonal antibodies against HBV surface antigens have been developed. Although the mouse monoclonal antibodies show high affinity for the antigen and can be prepared on a large scale, they induce human anti-mouse antibody response in patients (Shawler *et al., J. Immunol.*, 135:1530, 1985). There were attempts to prepare human monoclonal antibodies, but few of these antibodies showed a high level of affinity.

Instead, humanized antibodies have been developed. Humanized antibody has a high level of affinity and specificity similar to mouse antibodies, whereas its immunogenicity is minimized. Humanized antibody is a hybrid antibody in which CDRs (Complementarity Determining Regions) of a mouse antibody is grafted to

a human antibody by genetic engineering technique. It can be easily prepared on a large scale, and hardly elicits immune responses in humans since most of the DNA sequences encoding the humanized antibodies are derived from a human DNA sequence (Riechman *et al.*, *Nature*, 332:323, 1988; Nakatani *et al.*, *Protein Engineering*, 7:435, 1994).

To overcome the aforementioned and other disadvantages of mouse or human HBV immunoglobulin, we, the inventors of the present invention, have attempted to prepare humanized antibodies which can be used to prevent HBV infection and to treat chronic hepatitis B. Prior to this invention, we prepared a mouse monoclonal antibody KR127 against HBV surface antigen pre-S1. Additionally, we isolated the genes encoding the heavy and light chain variable regions of KR127 antibody and determined the sequences of the genes (Korea Patent Application No. 1997-30696). The present invention is performed by selecting human immunoglobulin genes homologous to the sequences of KR127 antibody light chain and heavy chain variable regions; constructing the humanized antibody genes; inserting the genes into expression vectors; introducing the vectors into host cells; obtaining humanized antibodies from the culture of the transformed cells; and confirming that the humanized antibodies have high affinity to HBV pre-S1

antigen, similar to the mouse monoclonal antibody KR127.

SUMMARY OF THE INVENTION

It is an object of this invention to provide humanized antibodies specific for CDRs of mouse HBV surface antigen pre-S1, having high affinity to the antigen and reduced immunogenicity in human.

In accordance with the present invention, the foregoing objects and advantages are readily obtained.

The present invention provides humanized antibodies specific for HBV surface antigen pre-S1, comprising humanized heavy and light chains.

This invention also provides genes encoding the variable regions of said humanized heavy or light chain.

In addition, this invention provides expression vectors containing said genes and *E. coli* transformants containing said expression vectors.

This invention further provides pharmaceutical compositions comprising said humanized antibody, which may be administered in order to prevent HBV infection or to treat chronic hepatitis B.

Further features of the present invention will appear hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG 1a and FIG 1b comparatively depict the amino acid and nucleotide sequences of V_H regions (for Variable regions of Heavy chains) in a mouse monoclonal antibody KR127 and in two humanized antibodies of this invention,

FIG 2a schematically depicts a process for preparing HKR127HC(I) gene encoding the heavy chain of a humanized antibody of this invention,

FIG 2b schematically depicts a process for preparing HKR127HC(III) gene encoding the heavy chain of a humanized antibody of this invention,

FIG 3a and FIG 3b comparatively depict the amino acid and nucleotide sequences of V_L regions (for Variable regions in Light chain) in a mouse monoclonal antibody KR127 and in a humanized antibody of this invention.

FIG 4 schematically depicts a process for preparing HKR127KC(I) gene encoding a humanized antibody of this invention,

FIG 5a depicts an expression vector pCMV-HKR127HC containing a gene for heavy chain of the humanized antibody,

FIG 5b depicts an expression vector pKC-dhfr-HKR127 containing a gene for light chain of the humanized antibody,

FIG 5c depicts an expression vector pCMV-HKR127HC(III) containing a gene for heavy chain of the humanized antibody,

FIG 6a comparatively shows the binding affinities of a humanized antibody (HZKR127I) and a mouse monoclonal antibody (KR127), and

FIG 6b comparatively shows the binding affinities of a humanized antibody (HZKR127III) and a humanized antibody (HZKR127I).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Hereinafter, the present invention is described in detail.

This invention provides humanized antibodies specific for HBV surface antigen pre-S1, comprising humanized heavy and humanized light chains.

This invention also provides genes encoding the variable regions of said humanized heavy or light chain.

Said humanized heavy chain contains variable region which is derived from the V_H region of mouse KR127 antibody. The V_H region of mouse KR127 antibody is described by SEQ ID NO: 19, and the V_H region of the humanized antibody of this invention can be prepared by grafting the CDRs of mouse KR127 V_H region to homologous human immunoglobulin V_H region.

And said humanized light chain contains variable

region which is derived from the V_L region of mouse KR127 antibody. The V_L region of mouse KR127 antibody is described by SEQ ID NO: 22, and the V_L region of the humanized antibody of this invention can be prepared by grafting the CDRs of mouse KR127 V_L region to homologous human immunoglobulin V_L region.

In preferred embodiments, we screened human immunoglobulin that show the highest similarities of amino acid sequence to the heavy or light chain of the mouse monoclonal antibody KR127. In result, human immunoglobulin germ line genes DP7 and DPK12 were screened from GenBank database. DP7 shows the highest homology to the V_H region of mouse antibody KR127, while DPK12 is most similar to the V_L region of KR127.

The humanized antibodies of this invention can be produced from recombinant genes encoding humanized V_H region or V_L region. These genes are constructed by substituting CDRs of mouse KR127 for those of the human DP7 or DPK12 antibody. In constructing these genes, most of the amino acid residues corresponding to the humanized CDRs are derived from the CDRs of mouse antibody KR127. However, some mouse-derived CDRs residues are replaced by human counterparts, since their corresponding amino acid residues are expected not to be involved in the antigen binding (see FIG 1). In the same way, some human-derived amino acid residues for the non-CDR framework regions (FR) of variable

region are replaced with mouse counterparts, since it is expected that these FR residues may affect the conformation of CDRs.

Particularly, HKR127HCv(HZII) gene encoding a humanized V_H region was prepared by grafting the partial CDR1, 2, 3 and a FR residue (at position 72) of mouse KR127 heavy chain to the human DP7 gene (see FIG 1).

However, antibody expressed from HKR127HCv(HZII) gene did not show any significant level of binding capacity to corresponding antigen. To improve the HKR127HCv(HZII) gene, we also prepared HKR127HCv(HZI) gene and HKR127HCv(HZIII) gene which contain more mouse-derived codons than HKR127HCv(HZII) gene (see FIG 1).

HKR127HCv(HZI) contains CDR1, partial CDR2, and CDR3, and 11 FR residues of mouse KR127 V_H, while HKR127HCv(HzIII) contains the same mouse CDR codons and 2 mouse FR residues (see FIG 1).

To construct HKR127HC(I) gene encoding a full-length heavy chain of the humanized antibody of this invention, PCRs were conducted, in which template for amplification is either the HKR127HCv(HZII) gene or pRC/CMV-HC-HuS (KCTC 0229BP) containing the heavy chain leader sequence and the constant region sequence of human immunoglobulin heavy chain γ1.

Six pairs of oligonucleotides (SEQ ID NO: 1 and 2;

3 and 4; 5 and 6; 7 and 8; 9 and 10; and 11 and 12) were used as PCR primers (see FIG 2a).

The first five PCR products were brought to annealing reaction. Then, the DNA fragment containing the five PCR products was employed as a template of recombinant PCR wherein two primers described by SEQ ID NO: 1 and 10 were used. Another recombinant PCR was conducted to link the amplified 431-bp DNA fragment to DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 11 and 12). The recombinant PCR employed two primers described by SEQ ID NO: 1 and 12. The final 1431-bp PCR product, HKR127HC(I), encoding the heavy chain of a humanized antibody (HZKR127I) was introduced into pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127HC(I).

The primers are described in SEQ ID NO: 1 to 12 in SEQUENCE LISTING, and particularly, primer described by SEQ ID NO: 1 contains EcoRI sequence at the 5' end, while primer described by SEQ ID NO: 12 does SalI sequence at the 3' end.

The variable region in the HKR127HC(I) gene contains 11 mouse-derived FR residues at positions 12, 28, 30, 48, 67, 68, 70, 72, 74, 79 and 95 (see FIG 1). The heavy chain variable region has 87 FR residues, and the unmodified FR residues is 76. Thus, the amino acid sequence of the heavy chain variable FR of the

HKR127HC(I) gene is 87% homologous to that of human DP7 gene.

To more humanize the HZKR127(I), HZKR127(III) gene was constructed, which contains HKR127HCv(HZIII) gene with 2 mouse-derived FR residues at position 72 and 74 (see FIG 1).

To construct HKR127HC(III) gene encoding a full-length heavy chain of the humanized antibody of this invention, PCRs were conducted, in which template for amplification is either the HKR127HCv(HZII) gene or pRC/CMV-HC-HuS (KCTC 0229BP) containing the heavy chain leader sequence and the constant region sequence of human immunoglobulin heavy chain γ1 (see FIG 2b).

Four pairs of oligonucleotides (SEQ ID NO: 1 and 24; 25 and 26; 27 and 28; and 11 and 12) were used as PCR primers (see FIG 2b). The first three PCR products were brought to annealing reaction. Then, the DNA fragment containing the three PCR products was employed as a template of recombinant PCR wherein two primers described by SEQ ID NO: 1 and 28 were used. Another recombinant PCR was conducted to link the amplified 431-bp DNA fragment to DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 11 and 12). The recombinant PCR employed two primers described by SEQ ID NO: 1 and 12.

The final 1431-bp PCR product, HKR127HC(III), encoding the heavy chain of a humanized antibody

(HZKR127III) was introduced into pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127HC(III).

In a further embodiment, HKR127KCv(HZII) gene encoding a humanized V_L region was prepared by grafting the CDR1, CDR3 and partial CDR2 of mouse KR127 light chain to the human DPK12 gene (see FIG 3).

However, antibody expressed by using the HKR127KCv(HZII) gene did not show any significant level of binding capacity to corresponding antigen. To improve the binding capacity of HKR127KCv(HZII), we also prepared HKR127KCv(HZI) gene which contains more mouse-derived amino acid residues (see FIG 3) than HKR127HKCv(HZII) (see FIG 3).

To construct HKR127KC(I) gene encoding a full-length light chain of the humanized antibody of this invention, PCRs were conducted, in which template for amplification is either the HKR127KCv(HZII) gene or pKC-dfhr-HuS (KCTC 0230BP) containing the light chain leader sequence and the constant region sequence of human immunoglobulin light chain κ.

Three pairs of oligonucleotides (SEQ ID NO: 13 and 14; 15 and 16; and 17 and 18) were used as PCR primers (see FIG 4).

The first two PCR products were brought to annealing reaction. Then, the DNA fragment containing

the two PCR products was employed as a template of recombinant PCR wherein two primers described by SEQ ID NO: 13 and 16 were used. Another recombinant PCR was conducted to link the amplified 360-bp DNA fragment to DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 17 and 18). The recombinant PCR employed two primers which are described by SEQ ID NO: 13 and 18. The final 739-bp PCR product, HKR127KC(I), encoding the light chain of a humanized antibody (HZKR127I) was introduced into pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127KC(I).

The primers are described in SEQ ID NO: 13 to 18 in SEQUENCE LISTING, and particularly, primer described by SEQ ID NO: 13 contains HindIII sequence at the 5' end, while primer described by SEQ ID NO: 18 does SalI sequence at the 3' end.

The variable region FR of the HKR127KC gene contains 5 mouse KR127-derived codons (see FIG 3). The light chain has 83 FR residues, and the unmodified FR residues is 78. Thus, the amino acid sequence of the light chain variable FR of the HKR127KC gene is 94% identical to that of human DP7 gene.

In addition, this invention provides expression vectors containing genes encoding the humanized V_H or V_L region and provides *E. coli* transformants containing

said expression vector.

In other preferred embodiments, expression vectors are prepared, which contain the gene encoding the heavy or light chain of humanized antibody (see FIG 5a, 5b or 5c).

Particularly, two kinds of DNA fragment corresponding to humanized heavy chain was respectively obtained from the plasmids pHKR127HC(I) and pHKR127HC(III) by treatment of restriction enzymes, and then inserted into pRc/CMV (Invitrogen) to give expression vector pCMV-HKR127HC (see FIG5a) and pCMV-HKR127(III)HC (see FIG 5c), respectively.

In addition, DNA fragment encoding the humanized light chain was isolated from the pHKR127KC vector, and then introduced into pCMV-dfhr (KCTC 8671P) to construct expression vector pKC-dhfr-HKR127 (see FIG 5b).

E. coli strain DH5α was transformed with the expression vector pCMV-HKR127HC, pCMV-HKR127(III)HC or pKC-dhfr-HKR127. The resulting *E. coli* transformants containing pCMV-HKR127HC or pKC-dhfr-HKR127 were deposited in KCTC (Korean Collection for Type Culture) (Accession Number: KCTC 0531BP and KCTC 0529BP, respectively) on October 12, 1998. The *E. coli* transformant containing pCMV-HKR127(III)HC was deposited in KCTC (Accession Number: KCTC 0691BP, respectively) on November 15, 1999.

In another preferred embodiment, humanized antibodies specific for HBV surface antigen pre-S1 were expressed in animal cells and obtained from culture media of the cells. COS7 cells were transiently cotransfected with the expression vectors pCMV-HKR127HC and pKC-dhfr-HKR127, and the resulting transfected cells was cultured and the culture supernatant was used to characterize a humanized antibody HZKR127I of the present invention. COS7 cells were also cotransfected with the expression vectors pCMV-HKR127(III)HC and pKC-dhfr-HKR127, and the culture supernatant of transfected cells was used to characterize a humanized antibody HZKR127III.

This invention further provides pharmaceutical compositions containing said humanized antibody.

According to still other preferred embodiments, it was verified that HZKR127I and HZKR127III humanized antibodies of the present invention, showed almost same antigen-binding affinity when compared with mouse monoclonal antibody KR127 (see Table 1, 2 and FIG 6a, 6b).

The composition includes a therapeutically effective amounts of the humanized antibody against HBV antigen pre-S1, with/without a pharmaceutically acceptable delivery vehicle. Moreover, the

compositions may include other anti-hepatitis drug(s), such as anti-S monoclonal antibody or lamivudin.

The humanized antibody against HBV antigen pre-S1 may be formulated with a pharmaceutical vehicle or diluent for intravenous, subcutaneous, intramuscular administration. The pharmaceutical composition can be formulated in a classical manner using solid or liquid vehicles, diluents and additives appropriate to the desired mode of administration.

The humanized antibody of this invention may be administered in a dosage range of about 1 ~ 10 mg/kg, preferably 3 ~ 5 mg/kg, and may be administered once a week.

EXAMPLES

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1: Preparation of gene encoding humanized heavy chain

In order to construct the humanized heavy chain variable region gene, first, we selected a human immunoglobulin heavy chain gene that shows the highest homology of amino acid sequence to the heavy chain variable region of the mouse monoclonal antibody KR127. As the result, a human immunoglobulin germ line gene DP7 was selected from GenBank database. Then, we constructed a humanized V_H region gene HKR127HCv(HZII) by DNA recombination techniques, which was based upon the comparison of the mouse KR127 V_H region with the human DP7 V_H region. Since the humanized heavy chain did not show significant antigen binding activity, we prepared HKR127HCv(HZI) gene encoding another V_H region in order to improve the HKR127HCv(HZII) gene (see FIG 1).

Particularly, the HKR127HCv(HZII) gene was constructed by grafting the V_H region of human DP7 gene with the partial CDR1, 2, and 3 and one FR residue at position 72 of mouse KR127 V_H region. It was assumed that the human CDRs and FR amino acid residues affected the antigen-binding affinity of the antibody.

Therefore, HKR127HCv(HZI) gene was constructed by PCR employing HKR127HCv(HZII) gene as a template.

On the other hand, a vector pRc/CMV-HC-HuS (Accession Number: KCTC 0229BP) was used to synthesize DNA sequence encoding human C_H region as well as heavy chain leader sequence, which is required in proper secretion of the heavy chain.

Finally, HKR127HC(I) gene encoding a humanized heavy chain was constructed by recombinant PCR for the annealing of the heavy chain leader sequence, HKR127HCv(HZI) gene, and the human C_H gene (see FIG 2a).

The primers in these PCRs are synthetic oligonucleotides described by SEQ ID NO: 1 to 12. PCR was performed by using Taq DNA polymerase, and its thermocycle was repeated 30 times, consisting of 1 minute at 94°C, 1 minute at 55°C, and then 1 minute at 72°C. Five pairs of oligonucleotides (SEQ ID NO: 1 and 2; 3 and 4; 5 and 6; 7 and 8; and 9 and 10) were used as PCR primers, and the five PCR products (113 bp; 96 bp; 120 bp; 78 bp; and 87 bp, respectively) were brought to annealing reaction. Then, the DNA fragments containing the five PCR products were employed as template of the recombinant PCR wherein primers described by SEQ ID NO: 1 and 10 were used. Another recombinant PCR was conducted to link the amplified 431-bp DNA fragment to 1015-bp DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 11 and 12). The recombinant PCR employed two primers described by SEQ ID NO: 1 and 12.

The final PCR product (HKR127HC(I), about 1431-bp) encoding a recombinant heavy chain of humanized antibody was introduced into the EcoRI-SalI site of pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127HC(I). The DNA sequence of the inserted gene was determined by dideoxynucleotide method.

To more humanize the HKR127HC(I), another humanized heavy chain gene, HKR127(III), which has much less number of mouse FR residues, was constructed.

To construct the HKR127HC(III), HKR127HCv(HZIII) gene was constructed by PCR employing HKR127HCv(HZII) gene as a template. On the other hand, a vector pRc/CMV-HC-HuS (Accession Number: KCTC 0229BP) was used to synthesize DNA sequence encoding human C_H region as well as heavy chain leader sequence, which is required in proper secretion of the heavy chain.

Finally, HKR127HC(III) gene encoding a humanized heavy chain was constructed by recombinant PCR for annealing of the heavy chain leader sequence, HKR127HCv(HZIII) gene, and the human C_H gene (see FIG 2b).

The primers in these PCRs are synthetic oligonucleotides described by SEQ ID NO: 24 to 28. PCR was performed by using Taq DNA polymerase, and its thermocycle was repeated 30 times, consisting of 1 minute at 94°C, 1 minute at 55°C, and then 1 minute at

72°C. Three pairs of oligonucleotides (SEQ ID NO: 1 and 24; 25 and 26; and 27 and 28) were used as PCR primers, and the three PCR products (179 bp; 141 bp; and 87 bp, respectively) were brought to annealing reaction. Then, the DNA fragments containing the three PCR products were employed as template of the recombinant PCR wherein primers described by SEQ ID NO: 1 and 28 were used. Another recombinant PCR was conducted to link the amplified 431-bp DNA fragment to 1015-bp DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 11 and 12). The recombinant PCR employed two primers described by SEQ ID NO: 1 and 12.

The final PCR product (HKR127HC(III), about 1431-bp) encoding a recombinant heavy chain of humanized antibody was introduced into the EcoRI-SalI site of pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127HC(III). The DNA sequence of the inserted gene was determined by dideoxynucleotide method.

Example 2: Preparation of gene encoding humanized light chain

In order to prepare humanized light chain containing variable region, we devised genes encoding

the light chain. First, we selected a human κ immunoglobulin gene that shows the highest homology of amino acid sequence to the light chain of the mouse monoclonal antibody KR127. As the result, a human κ immunoglobulin gene DPK12 was selected from GenBank database. Then, we constructed HKR127KCv(HZII) gene encoding a humanized V_L region by grafting CDR1, partial CDR2, and CDR3 and one FR residue at position 41 of the mouse KR127 V_L region to the human DPK12 V_L region. The resulting humanized V_L was not functional in antigen-binding. To improve the HKR127KCv(HZII) gene, we constructed HKR127KCv(HZI) gene encoding another V_L region (see FIG 3).

The HKR127KCv(HZI) gene was constructed by grafting the V_L region of human DPK12 antibody with a few FR residues and CDR1, CDR2 and CDR3 of mouse KR127 V_L (see FIG 3).

On the other hand, a vector pKC-dhfr-HuS (Accession Number: KCTC 0230BP) was used to synthesize DNA sequence encoding human C_L region as well as light chain leader sequence, which is required in proper secretion of the light chain.

Finally, HKR127KC(I) gene encoding a humanized light chain was prepared by recombinant PCR for the annealing of the PCR products, light chain leader sequence, the HKR127KCv(HZI) gene, and the human C_L gene (see FIG 4).

The primers in these PCRs are synthetic oligonucleotides described by SEQ ID NO: 13 to 18. The thermocycle of these PCRs was repeated 30 times, consisting of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C. Two pairs of oligonucleotides (SEQ ID NO: 13 and 14; and SEQ ID NO: 15 and 16) were used as PCR primers, and the two PCR products (101 bp and 159 bp, respectively) were brought to annealing reaction. Then, the DNA fragments containing the two PCR products was employed as a template of recombinant PCR wherein primers described by SEQ ID NO: 13 and 16 were used. Another recombinant PCR was conducted to link the amplified 248-bp DNA fragment to 515-bp DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 17 and 18). The recombinant PCR employed two primers described by SEQ ID NO: 13 and 18.

The final PCR product (HKR127KC(I), 736-bp) encoding a recombinant light chain of humanized antibody was introduced into the HindIII-SalI site of pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127KC(I). The DNA sequence of the inserted gene was determined by dideoxynucleotide method.

Example 3: Construction of expression vector containing the humanized heavy chain gene

The pHKR127HC(I) or pHKR127HC(III) plasmid of Example 1 was digested with SalI enzyme, and the both ends of the vector was made blunt using Klenow enzyme treatment. This DNA fragment was further digested with NotI enzyme to obtain the gene encoding humanized heavy chain.

On the other hand, pRc/CMV (Invitrogen) was cut with XbaI enzyme, and the ends of the vector was made blunt by treating with Klenow enzyme, and then digested with NotI.

The humanized heavy chain gene and the linearized vector were linked to give expression vector pCMV-HKR127HC or pCMV-HKR127(III)HC. The *E. coli* transformant containing pCMV-HKR127HC or pCMV-HKR127(III)HC was deposited in KCTC (Korean Collection for Type Culture) (Accession Number: KCTC 0531BP and KCTC 0691BP, respectively), and the expression vector pCMV-HKR127HC and pCMV-HKR127(III)HC is shown in FIG 5a and 5c, respectively.

Example 4: Construction of expression vector containing the humanized light chain gene

The pHKR127KC vector of Example 2 was digested with HindIII and ApaI enzymes, and the resulting fragment was inserted into HindIII-ApaI site of pCMV-dhfr (Accession Number: KCTC 8671P) to give expression vector pKC-dhfr-HKR127. The *E. coli* transformant containing pKC-dhfr-HKR127 was deposited in KCTC (Korean Collection for Type Culture) (Accession Number: KCTC 0529BP), and the expression vector pKC-dhfr-HKR127 is shown in FIG 5b.

Example 5: Expression of humanized antibody in COS7 cells

COS7 cells were maintained in DMEM (Gibco) supplemented by 10% calf serum at 37°C, under 5% CO_2 condition. The cells were inoculated in 100mm petri dishes, and then incubated at 37°C overnight.

To express a humanized antibody HZKR127I, 5 μg of pCMV-HKR127HC or pKC-dhfr-HKR127 was diluted with 800 μl of OPTI MEM I (Gibco), and 50 μl of Lipofectamin (Gibco) was also diluted with 800 μl of OPTI MEM I. These mixtures in 15-ml tubes were incubated at room temperature for 15 minutes or more. In the meantime,

COS7 cells were washed twice with OPTI MEM I.

OPTI MEM I (6.4 ml) was added to the DNA-Lipofectamin mixture, mixed well, and poured on the COS7 cells. After the cells were cultured in a CO_2 incubator for 72 hours, the medium was centrifuged, and the supernatant was concentrated by ultrafiltration kit. The concentration of antibody was determined by Sandwich ELISA using anti-human IgG and anti-human IgG-HRP (horseradish peroxidase) conjugate.

To express and obtain a humanized antibody HZKR127III, the same protocol was repeated except using pCMV-HKR127(III)HC instead of using pCMV-HKR127HC.

Example 6: Binding activity of humanized antibody to HBV surface antigen pre-S1

We prepared HBV surface antigen pre-S1 (amino acid residue 1-56; Kim and Hong, Biotechnology Letters, 17:871-876, 1995) and 1 μg of the purified pre-S1 was coated on each well in microplates. After addition of 0, 0.25, 0.5, 1, 2, 3, 4, 5, 7.5, 10, 20, or 40 ng of the humanized antibodies prepared in Example 5, indirect ELISA was performed, in which secondary antibody was Fc-specific anti-human IgG-HRP conjugate. The binding activities of the antibodies were determined by measuring OD at 492 nm.

Purified mouse KR127 antibody was used as a control, and ELISA of KR127 antibody was conducted using Fc-specific anti-mouse IgG-HRP conjugate as a secondary antibody. The result is presented in Table 1 and 2.

Table 1.

Binding activity of KR127 and HZKR127I to HBV surface antigen pre-S1 (OD at 492 nm)

Antibody \ Amount (ng)	0	0.25	0.5	1	2	3	4	5	7.5	10	20	40
KR127	0.09	0.12	0.15	0.20	0.30	0.36	0.43	0.54	0.60	0.80	1.16	1.64
HZKR127I	0.09	0.12	0.17	0.26	0.35	0.43	0.60	0.71	0.79	1.12	1.48	1.77

Table 2.

Binding activity of HZKR127I and HZKR127III to HBV surface antigen pre-S1 (OD at 492 nm)

Antibody \ Amount (ng)	0	0.25	0.5	1	2	3	4	5	7.5	10	20	40
HZKR127I	0.06	0.19	0.25	0.58	0.65	0.75	0.86	1.02	1.25	1.39	1.95	2.07
HZKR127III	0.06	0.20	0.37	0.60	0.87	1.10	1.24	1.37	1.65	1.89	2.04	2.10

Example 7: Antigen-binding affinity of humanized antibody to HBV surface antigen pre-S1

Antigen-binding affinity to HBV surface antigen

pre-S1 was assayed by competitive ELISA method(Ryu et al., J. Med. Virol., 52:226, 1997).

Binding reactions between the pre-S1 antigen ($1 \times 10^{-7} \sim 1 \times 10^{-12}$ M) and the humanized antibody of Example 5 (5 ng), or between the antigen ($1 \times 10^{-7} \sim 1 \times 10^{-12}$ M) and control antibody KR127 (5 ng), were performed at 37°C for 2 hours. Then the reaction mixtures were added to 96-well microplates coated with the 250 ng of antigen pre-S1.

FIG 6a shows the affinity of two kinds of antibodies. It was confirmed that the binding affinity of the humanized antibody HZKR127I is almost same as that of the mouse antibody KR127 (7×10^{7} M^{-1}).

FIG 6b shows the affinity of HZKR127III compared with that of HZKR127I. The affinity of HZKR127III (5×10^{7} M^{-1}) was not much different from that (7×10^{7} M^{-1}) of HZKR127I.

INDUSTRIAL APPLICABILITY

As shown above, the present invention provides humanized antibody against HBV surface antigen pre-S1, which shows similar level of binding affinity when compared with mouse monoclonal antibody, whereas immunogenicity of the humanized antibody is remarkably reduced. Thus, the humanized antibody of the present

invention may be useful for the prevention of HBV infection and for the treatment of hepatitis B.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO: Hong Hyo-Jeong
KIT Apt. 15-401, #237 Gajeong-dong, Yusong-ku, Taejon 305-350,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
***Escherichia coli* DH5α /pCMV-HER127EC**	**KCTC 0531BP**

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
[x] a scientific description
[ ] a proposed taxonomic designation
(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **October 12 1998**

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on .

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **Korean Collection for Type Cultures**	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	PARK Yong-Ha, Director Date: **October 17 1998**

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO: HONG, Hyo Jeong
KIT Apt. 15 401, #237, Kajeong-dong, Yusong-ku, Taejon 305-360,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Escherichia coli **DH5@/pCMV-HKR127(III)HC**	**KCTC 0691BP**

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
[x] a scientific description
[ ] a proposed taxonomic designation
(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **November 15 1999.**

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Korean Collection for Type Cultures	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s):
Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Bae K. S. BAE, Kyung Sook, Director Date: **November 18 1999**

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO: Hong Hyo-Jeong
KIT Apt. 15-401, #237 Gajeong-dong, Yusong-ku, Taejon 305-350,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: ***Escherichia coli* DH5α /pKC-dhfr-HKR127**	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **KCTC 0529BP**
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: [x] a scientific description [ ] a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **October 12 1998**	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: **Korean Collection for Type Cultures** Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  PARK Yong-Ha, Director Date: **October 17 1998**

What is Claimed is

1. **(deleted)**

2. **(amended)** A humanized antibody specific for HBV surface antigen pre-S1, containing humanized heavy chain variable region which comprises amino acid sequence described by SEQ ID NO: 20.

3. **(amended)** A humanized antibody specific for HBV surface antigen pre-S1, containing humanized heavy chain variable region which comprises amino acid sequence described by SEQ ID NO: 21.

4. **(amended)** The humanized antibody of claim 3, wherein the humanized heavy chain variable region comprises an amino acid sequence which is modified from an amino acid residue of SEQ ID NO: 21 by at least one amino acid substitution selected from the group comprising

Lys^{1} → Val^{1}, Thr^{28} → Ala^{28}, Thr^{30} → Ser^{30},

Met^{48} → Ile^{48}, Arg^{67} → Lys^{67}, Val^{68} → Ala^{68},

Met^{70} → Leu^{70}, Val^{71} → Ala^{71}, and Tyr^{93} → Phe^{93}.

5. (amended) A humanized antibody specific for HBV surface antigen pre-S1, containing humanized light chain variable region comprises amino acid sequence described by SEQ ID NO: 23.

6. A gene encoding humanized heavy chain which contains a humanized heavy chain variable region of claim 2, 3 or 4.

7. The gene of claim 6, wherein the humanized heavy chain variable region comprises amino acid sequence described by SEQ ID NO: 20.

8. The gene of claim 6, wherein the humanized heavy chain variable region comprises amino acid sequence described by SEQ ID NO: 21.

9. A gene encoding humanized light chain which contains a humanized light chain variable region comprising amino acid sequence described by SEQ ID NO: 23.

10. An expression vector containing the gene of claim 6.

11. The expression vector of claim 10, pCMV-HKR127HC, wherein the gene of claim 7 is inserted into pRC/CMV

(Accession Number: KCTC 0531BP).

12. The expression vector of claim 10, pCMV-HKR127(III)HC, wherein the gene of claim 8 is inserted into pRC/CMV (Accession Number: KCTC 0691BP).

13. An expression vector containing the gene of claim 9.

14. The expression vector of claim 13, pKC-dhfr-HKR127, wherein the gene of claim 9 is inserted into pCMV-dhfr (Accession Number: KCTC 0529BP).

15. (amended) Pharmaceutical composition containing the humanized antibody of claim 2,3,4 or 5, which may be administered in order to prevent HBV infection or to treat chronic hepatitis B.

FIG. 1a

	Q	V	Q	L	Q	Q	S	G	P	E	L	V	K	P	
KR127VH	CAG	GTC	CAG	CTG	CAG	CAG	TCT	GGA	CCT	GAA	CTG	GTG	AAG	CCT	42
DP7	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCT	GAG	GTG	AAG	AAG	CCT	
HZII	CAG	GTC	CAG	CTG	GTG	CAG	TCT	GGA	GCT	GAA	GTG	AAG	AAG	CCT	
HZI	CAG	GTC	CAG	CTG	GTG	CAG	TCT	GGA	GCT	GAA	GTG	GTG	AAG	CCT	
HZIII	CAG	GTC	CAG	CTG	GTG	CAG	TCT	GGA	GCT	GAA	GTG	AAG	AAG	CCT	42
HZII	-	-	-	-	V	-	-	-	A	-	V	K	-	-	
HZI	-	-	-	-	V	-	-	-	A	-	V	-	-	-	
HZIII	-	-	-	-	V	-	-	-	A	-	V	K	-	-	

	G	A	S	V	K	I	S	C	K	A	S	G	Y	A	
KR127VH	GGG	GCC	TCA	GTG	AAG	ATT	TCC	TGC	AAA	GCT	TCT	GGC	TAC	GCA	84
DP7	GGG	GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAG	GCA	TCT	GGA	TAC	ACC	
HZII	GGG	GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAA	GCT	TCT	GGC	TAC	ACC	
HZI	GGG	GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAA	GCT	TCT	GGC	TAC	GCA	
HZIII	GGG	GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAA	GCT	TCT	GGC	TAC	ACC	84
HZII	-	-	-	-	-	V	-	-	-	-	-	-	-	T	
HZI	-	-	-	-	-	V	-	-	-	-	-	-	-	-	
HZIII	-	-	-	-	-	V	-	-	-	-	-	-	-	T	

	F	S	S	S	W	M	N	W	V	K	Q	R	P	G	
KR127VH	TTC	AGT	AGT	TCT	TGG	ATG	AAC	TGG	GTG	AAG	CAG	AGG	CCT	GGA	126
DP7	TTC	ACC	AGC	TAC	TAT	ATG	CAC	TGG	GTG	CGA	CAG	GCC	CCT	GGA	
HZII	TTC	ACC	AGT	TAC	TGG	ATG	AAC	TGG	GTG	CGA	CAG	GCC	CCT	GGA	
HZI	TTC	AGT	AGT	TCT	TGG	ATG	AAC	TGG	GTG	CGA	CAG	GCC	CCT	GGA	
HZIII	TTC	ACC	AGT	TCT	TGG	ATG	AAC	TGG	GTG	CGA	CAG	GCC	CCT	GGA	
HZII	-	T	-	Y	-	-	-	-	-	R	-	A	-	-	
HZI	-	-	-	-	-	-	-	-	-	R	-	A	-	-	
HZIII	-	T	-	-	-	-	-	-	-	R	-	A	-	-	

	Q	G	L	E	W	I	G	R	I	Y	P	G	D	G	
KR127VH	CAG	GGT	CTT	GAG	TGG	ATT	GGA	CGG	ATT	TAT	CCT	GGA	GAT	GGA	168
DP7	CAA	GGG	CTT	GAG	TGG	ATG	GGA	ATA	ATC	AAC	CCT	AGT	GGT	GGT	
HZII	CAG	GGT	CTT	GAG	TGG	ATG	GGA	CGG	ATT	TAT	CCT	GGA	GAT	GGA	
HZI	CAG	GGT	CTT	GAG	TGG	ATT	GGA	CGG	ATT	TAT	CCT	GGA	GAT	GGA	
HZIII	CAG	GGT	CTT	GAG	TGG	ATG	GGA	CGG	ATT	TAT	CCT	GGA	GAT	GGA	
HZII	-	-	-	-	-	M	-	-	-	-	-	-	-	-	
HZI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
HZIII	-	-	-	-	-	M	-	-	-	-	-	-	-	-	

	D	T	N	Y	N	G	K	F	K	G	K	A	T	L	
KR127VH	GAT	ACT	AAC	TAC	AAT	GGG	AAG	TTC	AAG	GGC	AAG	GCC	ACA	CTG	210
DP7	AGC	ACA	AGC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AGA	GTC	ACC	ATG	
HZII	GAT	ACT	AAC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AGA	GTC	ACA	ATG	
HZI	GAT	ACT	AAC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AAG	GCC	ACA	CTG	
HZIII	GAT	ACT	AAC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AGA	GTC	ACA	ATG	
HZII	-	-	-	-	A	Q	-	-	Q	-	R	V	-	M	
HZI	-	-	-	-	A	Q	-	-	Q	-	-	-	-	-	
HZIII	-	-	-	-	A	Q	-	-	Q	-	R	V	-	M	

FIG. 1b

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            T   A   D   K   S   S   S   T   A   Y   M   Q   L   S
KR127VH     ACT GCA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC AGC 252
DP7         ACC AGG GAC ACG TCC ACG AGC ACA GTC TAC ATG GAG CTG AGC
HZII        ACT GCA GAC ACG TCC ACG AGC ACA GTC TAC ATG GAG CTC AGC
HZI         ACC GCA GAC AAA TCC ACG AGC ACA GCC TAC ATG GAG CTG AGC
HZIII       ACT GCA GAC AAA TCC ACG AGC ACA GTC TAC ATG GAG CTC AGC
HZII        -   -   -   T   -   T   -   -   V   -   -   E   -   -
HZI         -   -   -   -   -   T   -   -   -   -   -   E   -   -
HZIII       -   -   -   -   -   T   -   -   V   -   -   E   -   -

            S   L   T   S   V   D   S   A   V   Y   F   C   A   R
KR127VH     AGC CTG ACC TCT GTG GAC TCT GCG GTC TAT TTC TGT GCA AGA 294
DP7         AGC CTG AGA TCT GAG GAC ACG GCC GTG TAT TAC TGT GCG AGA
HZII        AGC CTG AGA TCT GAG GAC ACG GCG GTC TAT TAC TGT GCA AGA
HZI         AGC CTG AGA TCT GAG GAC ACG GCG GTC TAT TTC TGT GCA AGA
HZIII       AGC CTG AGA TCT GAG GAC ACG GCG GTC TAT TAC TGT GCA AGA
HZII        -   -   R   -   E   -   T   -   -   -   Y   -   -   -
HZI         -   -   R   -   E   -   T   -   -   -   Y   -   -   -
HZIII       -   -   R   -   E   -   T   -   -   -   Y   -   -   -

            E   Y   D   E   A   Y   W   G   Q   G   T   L   V   T
KR127VH     GAG TAC GAC GAG GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT 336
HZII        GAG TAC GAC GAG GAC TAC TGG GGC CAA GGG ACT CTG GTC ACT
HZI         GAG TAC GAC GAG GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT
HZIII       GAG TAC GAC GAG GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT
HZII        -   -   -   -   D   -   -   -   -   -   -   -   -   -
HZI         -   -   -   -   -   -   -   -   -   -   -   -   -   -
HZIII       -   -   -   -   -   -   -   -   -   -   -   -   -   -

            V   S   A
KR127VH     GTC TCT GCA 345

HZII        GTC TCT TCA
HZI         GTC TCT TCA
HZIII       GTC TCT TCA
HZII        -   -   S
HZI         -   -   S
HZIII       -   -   S
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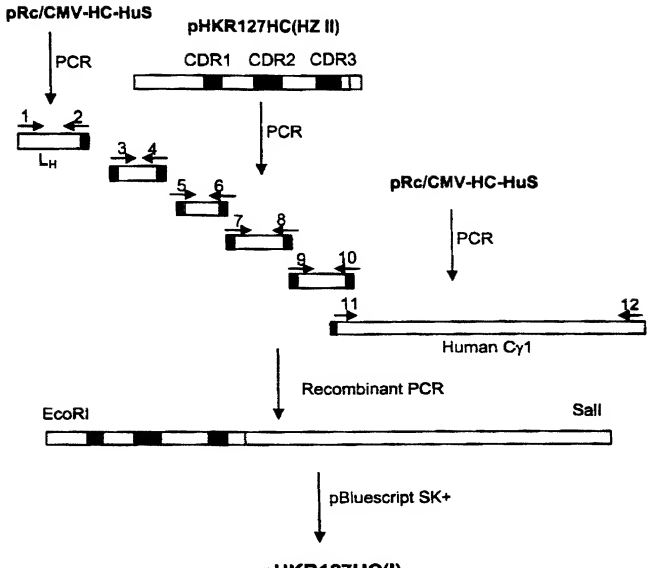
FIG. 2a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PCR
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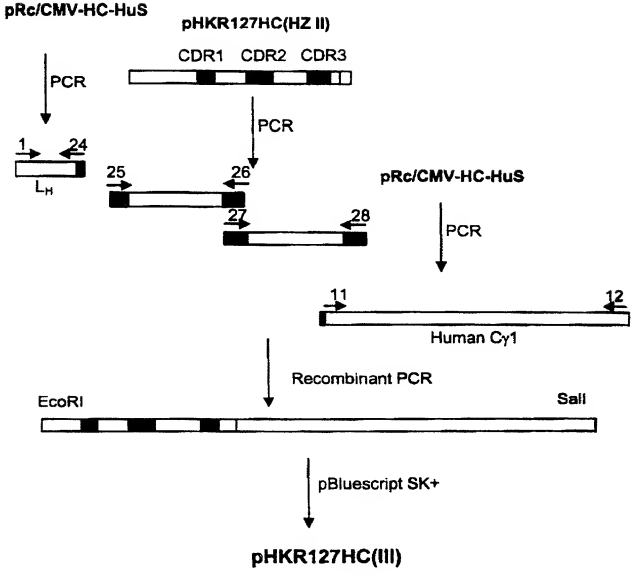
2
L_H
3
4
5
6
7
8
9
10
pRc/CMV-HC-HuS
PCR
11
12
Human Cγ1
Recombinant PCR
EcoRI
SalI
pBluescript SK+
pHKR127HC(I)

FIG. 2b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pHKR127HC(HZ II)
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PCR
PCR
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24
L_H
25
26
27
28
pRc/CMV-HC-HuS
PCR
11
12
Human Cγ1
Recombinant PCR
EcoRI
SalI
pBluescript SK+
pHKR127HC(III)

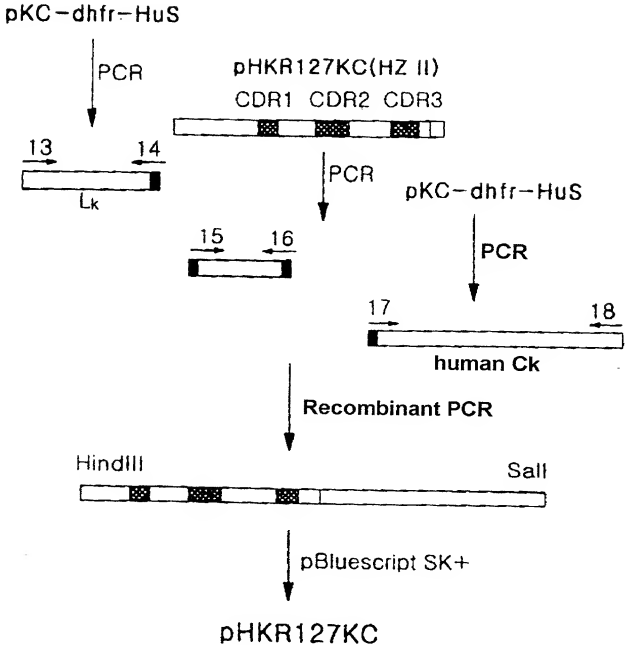
FIG. 3a

```
          D   I   L   M   T   Q   T   P   L   I   L   S   V   T
KR127VK GAT ATC TTG ATG ACC CAA ACT CCA CTT ATT TTG TCG GTT ACC 42
DPK12   GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC GTC ACC
HZII    GAT ATC GTG ATG ACC CAA ACT CCA CTT TCT TTG TCG GTT ACC
HZII    -   -   V   -   -   -   -   -   -   S   -   -   -   -
HZI     -   -   -   -   -   -   -   -   -   S   -   -   -   -

          I   G   Q   P   A   S   I   S   C   K   S   S   Q   S
KR127VK ATT GGA CAA CCA GCC TCT ATC TCT TGC AAG TCA AGT CAG AGC 84
DPK12   CCT GGA CAG CCG GCC TCC ATC TCC TGC AAG TCT AGT CAG AGC
HZII    CCT GGA CAA CCA GCC TCT ATC TCT TGC AAG TCA AGT CAG AGC
HZII    P   -   -   -   -   -   -   -   -   -   -   -   -   -
HZI     P   -   -   -   -   -   -   -   -   -   -   -   -   -

          L   L   Y   S   N   G   K   T   Y   L   N   W   L   L
KR127VK CTC TTA TAT AGT AAT GGA AAA ACC TAT TTG AAT TGG TTA TTA 126
DPK12   CTC CTG CAT AGT GAT GGA AAG ACC TAT TTG TAT TGG TAC CTG
HZII    CTC TTA TAT AGT AAT GGA AAA ACC TAT TTG AAT TGG TTA TTA
HZII    -   -   -   -   -   -   -   -   -   -   -   -   -   -
HZI     -   -   -   -   -   -   -   -   -   -   -   -   -   -

          Q   R   P   G   Q   S   P   K   R   L   I   Y   L   V
KR127VK CAG AGG CCA GGC CAG TCT CCA AAG CGC CTA ATC TAT CTG GTG 168
DPK12   CAG AAG CCA GGC CAG CCT CCA CAG CTC CTG ATC TAT GAA GTT
HZII    CAG AAG CCA GGC CAG CCT CCA CAG CTC CTA ATC TAT CTG GTG
HZII    -   K   -   -   -   P   -   Q   L   -   -   -   -   -
HZI     -   K   -   -   -   -   -   -   -   -   -   -   -   -

          S   K   L   D   S   G   V   P   D   R   F   T   G   S
KR127VK TCT AAA CTG GAC TCT GGA GTC CCT GAC AGG TTC ACT GGC AGT 210
DPK12   TCC AAC CGG TTC TCT GGA GTG CCA GAT AGG TTC AGT GGC AGC
HZII    TCT AAA CGG TTC TCT GGA GTC CCT GAC AGG TTC AGT GGC AGT
HZII    -   -   R   F   -   -   -   -   -   -   -   S   -   -
HZI     -   -   -   -   -   -   -   -   -   -   -   S   -   -
```

FIG. 3b

```
          G   S   G   T   D   F   T   L   K   I   I   R   V   E
KR127VK GGA TCA GGA ACA GAT TTT ACA CTG AAA ATC ATC AGA GTG GAG 252
DPK12   GGG TCA GGG ACA GAT TTC ACA CTG AAA ATC AGC CGG GTG GAG
HZII    GGA TCA GGA ACA GAT TTT ACA CTG AAA ATC AGC AGA GTG GAG
HZII    -   -   -   -   -   -   -   -   -   -   S   -   -   -
HZI     -   -   -   -   -   -   -   -   -   -   S   -   -   -

          A   E   D   L   G   V   Y   Y   C   V   Q   G   T   H
KR127VK GCT GAG GAT TTG GGA GTT TAT TAC TGC GTG CAA GGT ACA CAT 294
DPK12   GCT GAG GAT GTT GGG GTT TAT TAC TGC ATG CAA AGT ATA CAG
HZII    GCT GAG GAT GTT GGA GTT TAT TAC TGC GTG CAA GGT ACA CAT
HZII    -   -   -   V   -   -   -   -   -   -   -   -   -   -
HZI     -   -   -   V   -   -   -   -   -   -   -   -   -   -

          F   P   Q   T   F   G   G   G   T   K   L   E   I   K
KR127VK TTT CCT CAG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA 336
DPK12   CTT CCT CC
HZII    TTT CCT CAG ACG TTC GGT GGA GGC ACC AAG GTG GAA ATC AAA
HZII    -   -   -   -   -   -   -   -   -   -   V   -   -   -
HZI     -   -   -   -   -   -   -   -   -   -   V   -   -   -

          R
KR127VK CGG 339

HZII    CGG
HZII    -
HZI     -
```

FIG. 4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PCR
pKC-dhfr-HuS
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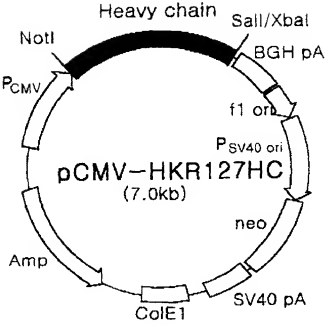
16
PCR
17
18
human Ck
Recombinant PCR
HindIII
SalI
pBluescript SK+
pHKR127KC

FIG. 5a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NotI
SalI/XbaI
BGH pA
P_{CMV}
f1 ori
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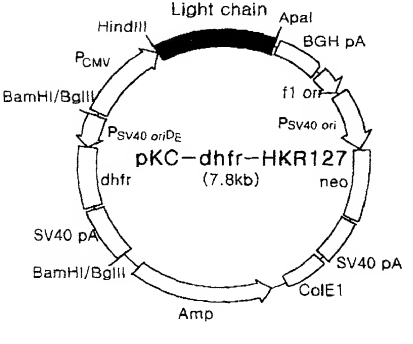
pCMV-HKR127HC
(7.0kb)
neo
Amp
ColE1
SV40 pA

FIG. 5b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HindIII
ApaI
BGH pA
P_{CMV}
BamHI/BglII
f1 ori
$P_{SV40\ ori}$
$P_{SV40\ ori D_E}$
pKC-dhfr-HKR127
dhfr
(7.8kb)
neo
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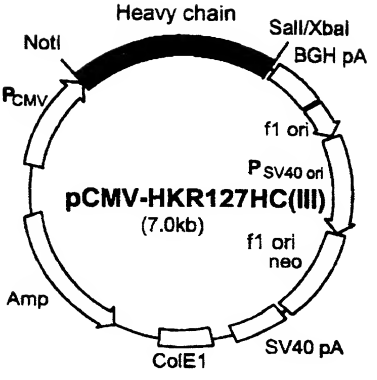
SV40 pA
ColE1
Amp

FIG. 5c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BGH pA
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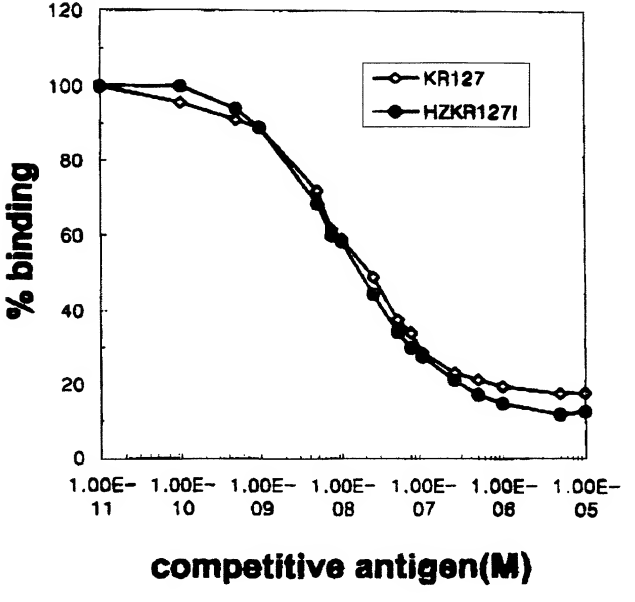
(7.0kb)
f1 ori
neo
Amp
ColE1
SV40 pA

FIG. 6a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80
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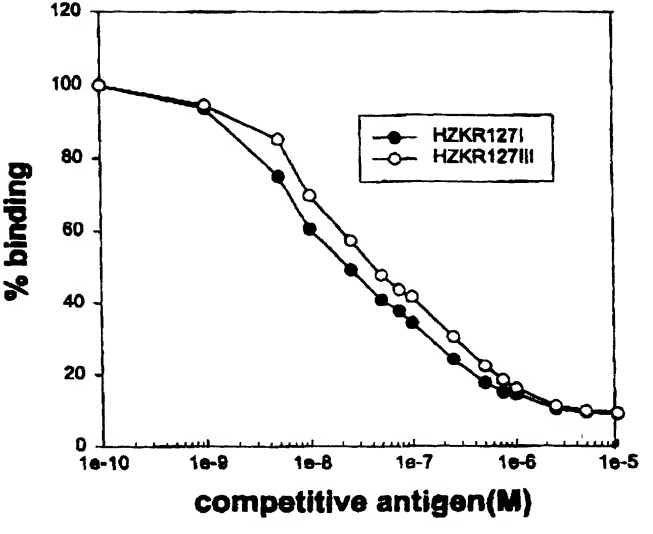
1.00E-09
1.00E-08
1.00E-07
1.00E-06
1.00E-05
competitive antigen(M)

FIG. 6b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80
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40
20
0
% binding
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HZKR127III
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1e-9
1e-8
1e-7
1e-6
1e-5
competitive antigen(M)

GATES & COOPER LLP

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention
entitled:
HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF HBV
AND PREPARATION METHOD THEREOF

The specification of which was filed on November 19, 1999 as PCT International Application Number
(INTERNATIONAL FILING DATE)
PCT/KR99/00699
(INTERNATIONAL APPLICATION NUMBER), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, ° 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, ° 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT application having a filing date before that of the application on the basis of which priority is claimed:

a. ☐ no such applications have been filed.
b. ☐ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC ° 119			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
KR	1998-49663	November 19, 1998	
OTHER FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

I hereby claim the benefit under Title 35, United States Code, ° 120 of any United States application(s), or 365(c) of any PCT international application(s) designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, ° 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, ° 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. PARENT APPLICATION OR PCT PARENT NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)

I hereby claim the benefit under Title 35, United States Code ° 119(e) of any United States provisional application(s) listed below:

U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)

I hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

George H. Gates Registration No. 33,500
Victor G. Cooper Registration No. 39,641
Karen S. Canady Registration No. 39,927
William J. Wood Registration No. 42,236
Jason S. Feldmar Registration No. 39,187
Bradley K. Lortz Registration No. 45,472

I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Gates & Cooper LLP to the contrary.

Please direct all correspondence in this case to the firm of Gates & Cooper LLP at the address indicated below:

~~GATES & COOPER LLP~~
~~Howard Hughes Center~~
6701 Center Drive West, Suite 1050
~~Los~~ Angeles, CA 90045

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(1)	Full Name Of Inventor	Family Name HONG	First Given Name Hyo Jeong	Second Given Name
	Residence & Citizenship	City ~~Taejon-si~~	State or Foreign Country Republic of Korea	Country of Citizenship Republic of Korea
	Post Office Address	Post Office Address #15-401 KIT Apt., 237 Kajeong-dong, Eusong-ku	City Taejon-si	State & Zip Code/Country 305-350/KR
Signature of Inventor(1): H. J. Hong				Date: April 20, 2001

(G&C)

PCT ô U.S. National Stage

(2) Full Name Of Inventor	Family Name RYU		First Given Name Chun Jeih	Second Given Name
Residence & Citizenship	City ~~Taejon-si~~	KR	State or Foreign Country Republic of Korea	Country of Citizenship Republic of Korea
Post Office Address	Post Office Address #136-1203 Hanbit Apt., 99 Oeun-dong, Eusong-ku,		City Taejon-si	State & Zip Code/Country 305-333/KR
Signature of Inventor(2): Chun Jeih Ryu				Date: April 20, 2001
(3) Full Name Of Inventor	Family Name HUR		First Given Name Hyangsuk	Second Given Name
Residence & Citizenship	City ~~Taejon-si~~	KR	State or Foreign Country Republic of Korea	Country of Citizenship Republic of Korea
Post Office Address	Post Office Address #106-1508 Expo Apt., Jeonmin-dong, Eusong-ku		City Taejon-si	State & Zip Code/Country 305-390/KR
Signature of Inventor(3): Hyangsuk				Date: April 20, 2001

º 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by °° 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) it establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) it refutes, or is inconsistent with, a position the applicant takes in:

(i) opposing an argument of unpatentability relied on by the Office, or

(ii) asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) each inventor named in the application:

(2) each attorney or agent who prepares or prosecutes the application; and

(3) every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

SEQUENCE LISTING

<110> Hyo Jeong Hong
Chun Jeih Ryu
Hangsook Hur

<120> HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF HBV AND PREPARATION METHOD THEREOF

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Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Phe Cys
85 90 95
Ala Arg Glu Tyr Asp Glu Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110
Val Ser Ala
115

<210> 20
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<212> PRT
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<220>
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HKR127HC(HZI)

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1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95
Ala Arg Glu Tyr Asp Glu Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110
Val Ser Ser
115

<210> 21
<211> 115
<212> PRT
<213> Artificial Sequence

<220>
<223> Variable region of humanized heavy chain
HKR127HC(HZIII)

<400> 21
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Ser
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Ala Gln Lys Phe
50 55 60
Gln Gly Arg Val Thr Met Thr Ala Asp Lys Ser Thr Ser Thr Val Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Glu Tyr Asp Glu Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110
Val Ser Ser
115

<210> 22
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Variable region of light chain in mouse KR127
antibody

<400> 22
Asp Ile Leu Met Thr Gln Thr Pro Leu Ile Leu Ser Val Thr Ile Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30
Asn Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
35 40 45
Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ile Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Val Gln Gly
                85                  90                  95
Thr His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
Arg
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<210> 23
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Variable region of humanized light chain

<400> 23
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 1               5                  10                  15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30
Asn Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
                85                  90                  95
Thr His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
Arg
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<211> 27
<212> DNA
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<220>
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<400> 24
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<210> 25
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<220>
<223> Synthetic oligonucleotide primer 25

<400> 25
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<210> 26
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<220>
<223> Synthetic oligonucleotide primer 26

<400> 26
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<210> 27
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<220>
<223> Synthetic oligonucleotide primer 27

<400> 27
gacaaatcca cgagcacagt ctacatg 27

<210> 28
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<400> 28
gtcgtactct ctcgcacagt aatacac 27